THE SPERMATOGENESIS OF ONISCUS ASELLUS LINN.[1] WITH ESPECIAL REFERENCE TO THE HISTORY OF THE CHROMATIN.[2]

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(Plates XI-XVIII.)

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This study was begun in the month of February, 1899, in order to ascertain the mode of origin of the peculiar spermatozoa of the land Isopods. I have now completed, so far as I am able at the present time, the investigation undertaken for that purpose. Before entering, however, upon a description of my observations, I wish, at the close of a work which has proved both interesting and instructive, to express my gratitude to my instructors, Prof. E. G. Conklin and Prof. Thomas H. Montgomery, Jr., for the inspiration and the many valuable suggestions which have aided me toward its completion. To the latter I am particularly indebted for his helpful criticism concerning the earlier stages of the spermatogenesis.

METHODS.

The material was fixed either in Flemming's fluid, Hermann's fluid or in Gilson's fluid (acetic-nitric sublimate). It was stained for the most part with iron hæmatoxylin, but for purposes of comparison also with saffranin and malachite green (Wilcox) (1895), saffranin and gentian violet, Delafield's hæmatoxylin and Bordeaux red, and with the Biondi-Ehrlich triple stain. The study of the spermatozoa was also pursued by teasing apart the vas deferens with needles, staining the fresh material with hæmatoxylin or with acetic-methyl green, and mounting in glycerin. Permanent mounts were also made of fixed and stained material. I consider Wilcox's double stain with saffranin and malachite green to be a valuable one, for the reason that it can be used with good effect on material fixed in Flemming's fluid. It gives in reality a triple stain, for in successful preparations the cytoplasmic structures stain green, active chromatin, centrosomes and true nucleoli red, while resting chromatin takes a purple color. Its chief disadvantage is that it will in time fade.

[1] The species was determined by means of the works of Budde-Lund (1885) and of Richardson (1900). The species is also known as O. murarius, Cuv.

[2] A thesis for the degree of Ph.D. at the University of Pennsylvania.

I. Structure of the Male Reproductive Organs.

The male reproductive organs of the land Isopods are paired and lie on either side of the tubular intestine, occupying almost the entire length of the thoracic region.

The testis consists of three narrow lobes, which are attached to the body musculature by slight strands of tissue. These lobes are distinct from each other and open successively into the anterior expanded portion of the vas deferens (Fig. 1). Posteriorly the vas deferens narrows to a more slender tube, which joins its fellow of the opposite side and opens through the penis, which is unpaired and is said by Gerstaecker (1882) to be an outgrowth from the seventh thoracic segment. It is enclosed by the modified internal lamellæ of the first abdominal appendages (Fig. 1).

Sections of the vas deferens (Fig. 3) show its expanded portion to be lined with cells of large size, which possess prominent spherical nuclei. The nucleus is sometimes surrounded by a clear space, varying somewhat in size. The chromatin is in the form of closely crowded granules. Between these are sometimes other granules, which with the iron hæmatoxylin stain are less deeply colored, and with Bordeaux red and Delafield's hæmatoxylin take a red tint. The periphery of the latter is usually darker in color. The surrounding cytoplasm is filled with particles of a rounded shape, which take cytoplasmic stains (Fig. 4*a*). In one preparation the cytoplasm of these cells was filled with particles, not rounded in shape but thread-like, and taking a very dark stain with iron hæmatoxylin. The chromatin consisted of granules of varying size, which appeared lighter in the centre and possessed a darker margin (Fig. 4*b*). I do not know whether there is any connection between the particles within the nucleus and those without; the subject might possibly repay further research. The appearance of these cells suggests strongly that they have a secretory function; no doubt the fluid which bathes the spermatozoa is produced by them. They are more abundant at those places where the follicles open into the vas deferens and grow more scarce in the region where the narrow portion of the vas commences (Fig. 3). Between them are to be seen nuclei of smaller size, whose chromatin is not so distinctly and regularly granular. These lie in a cytoplasmic reticulum of a coarse mesh, without well-defined cell boundaries and containing no granules. This tissue apparently forms a supporting

membrane for the secretory cells. It is continuous with the layer of cells which line the narrow portion of the vas deferens and is similar to it in structure (Fig. 3). The narrow portion of the vas, as a rule, is covered externally by dark pigment, thus forming a marked contrast in the fresh state to the milk-white walls of the anterior portion (Fig. 1). Between the pigment layer and the lining cells, delicate muscle-fibres are occasionally discernible.

The three lobes of the testis are seen in section to be three follicles (Fig. 2). Each follicle is covered by a thin membrane which is provided with delicate muscle-fibres (Fig. 5*m*, *l*). The margins of the follicle are occupied by large nuclei of unsymmetrical outline, containing irregular blocks of chromatin interspersed with finer granules. Cell boundaries between these nuclei are not visible. They can sometimes be seen to be undergoing amitotic division, of a character similar to that described by vom Rath (1891) for Astacus (Fig. 5*f*, *c*). In follicles of a certain stage of development these nuclei, as will be explained later, are subject to degeneration.

The strands of tissue, by means of which the follicles are suspended from the body wall, are made up of cells which also divide amitotically and which are similiar in appearance to the follicle nuclei, inasmuch as their outlines are irregular, but the blocks of chromatin are of larger size and the nuclei are separated from each other by distinct cell walls (Fig. 6).

The interior of the follicle, except during the migration of the follicle cells, is occupied entirely by the germ cells, which are in differing stages of development in the three follicles of one side. Corresponding follicles of opposite sides of the body contain, however, germ cells which have developed to very nearly the same degree.

Fig. 2 shows, in a typical case, the comparative degrees of development to which the cells of the three follicles have attained. Each follicle may be divided into two principal regions of growth, composed of cells of different generations and of different degrees of development. Thus, in the most posterior of the follicles (*a*), the apical third is occupied exclusively by spermatogonia, some of which can be seen in mitosis; the basal region, on the other hand, by spermatids in a not very advanced stage. Follicle cells occur on the outside of the follicle, being especially abundant in the basal region. In the adjacent follicle (*b*), the apical two-thirds is occupied by cells in the synapsis stage, the remaining portion by sper-

matids in a stage of development later than that of follicle (*a*). Along the margin of the follicle are found scattered small groups of spermatagonia (Fig. 2, *spg*). The third and most anterior follicle (*c*) contains chiefly spermatocytes in a late prophase. Groups of spermatogonia similiar to those of follicle (*b*) are here also found scattered along the margin and nearly filling the extreme apical portion. The follicle cells in the basal region are undergoing not only active amitotic division, but to a certain extent degeneration (Fig. 5). Their active multiplication or fragmentation causes them to crowd in toward the axis of the follicle.

From a comparison of the extent of these growth regions in the three follicles, the developmental cycle may be conceived somewhat as follows. The spermatozoa, when fully formed, are forced into the vas deferens. Since they have no motion of their own, this is probably caused by the contraction of the muscle layer of the follicle, perhaps assisted by the pressure of the growing cells in the apical region. During this process, the spermatogonia in the apical portion of the follicle divide and come to fill the space left vacant by the discharged sperm. The rate at which this replacement takes place and the comparative development of the cells in the two regions may vary in different follicles, for I have preparations in which few or no spermatozoa are seen—in other words, most of them had probably been discharged, and at the same time the replacing spermatogonia are scattered and few in number. In others, as is shown in the diagram (Fig. 2*a*), the spermatozoa, in an early stage of development, occupy the basal portion of the follicle, while the apical portion is packed with spermatogonia. The majority of the spermatogonia thus filling up the follicle proceed in their development, while the remainder form the groups of cells along the margin of the follicle already described in follicles (*b*) and (*c*), and which are destined later to again supply a new generation of cells. The spermatids also proceed in development and are forced into the vas deferens. A condition like that represented in diagram (*b*) (Fig. 2) thus arises—the basal region filled with spermatozoa in a late stage about to pass into the vas deferens and the apical region with cells which have progressed as far as the synapsis stage. Later, the spermatozoa having been completely discharged, the cells of the apical region come to occupy the basal part of the follicle, being now less compactly pressed together (Fig. 2*c*). Their development progresses until, having become mature spermatozoa, they pass into

the vas deferens, the spermatogonia again fill the apical region, and the cycle is repeated.

The invasion of the follicle cells begins, as a rule, when the germ cells are in an advanced prophase and may continue later. Many of the germ cells likewise degenerate, and they, together with the follicle cells, form a disintegrated mass in which the spermatids lie. In young follicles, which have not as yet matured sperm, the basal region is filled with follicle cells, the apical region with spermatogonia. This is sometimes true also of older follicles which have recently discharged the sperm.

It will thus be seen that a series of stages, illustrating the complete history of the changes through which the germ cell pass, can be obtained only by an examination of numerous testes. Duplicates are often obtained and some of the stages occur very infrequently, probably owing to a greater rapidity of development at certain periods.

This study was begun in the latter part of February. In March or April, according to the rigor of the weather, the land Isopods in the vicinity of Philadelphia commence to breed. The breeding season continues during the summer months. There are, in a single year, several cycles of development of the reproductive elements; the exact number I have not determined. It is therefore possible, at almost any time of year, by examination of a sufficient number of individuals, to procure a complete series of developmental stages.

II. Spermatogenesis.

1. Spermatogonia.

The resting spermatogonia are distinguished from the follicle cells by their smaller size, the distinctness of the cell walls, and by the fact that in their nuclei the chromatin masses are of smaller size and show indications of an arrangement into a network (Fig. 11*a*). They possess a prominent true nucleolus of more or less rounded form. Some cells contain one or more smaller nucleoli.

It is impossible to determine the exact number of spermatogonic divisions. They are probably numerous, since it must require a considerable number of divisions of the spermatogonia remaining in the follicles to fill the space left vacant by the discharged sperm. The cells vary somewhat in size. When the apical region of the follicle is filled with spermatogonia the individual cells are small, but when the follicle is not well filled and the spermatogonia are

beginning the task of producing a new generation, individual cells often equal the spermatocytes in size (Figs. 10 and 13). In the cytoplasm are occasionally seen irregular masses of a dull brown tint (yolk ?), but neither sphere substance nor centrosomes are apparent in the resting cell.

In nuclei preparing to divide, the chromatin is seen to be arranged in the form of slender, elongated threads, which, so far as I have been able to discover, in no case form a continuous spireme (Fig. 7). In the cytoplasm surrounding a nucleus of this character are visible two minute black specks joined by a delicate thread, presumably the centrosome undergoing division. The nuclear membrane at this stage begins to fade. Figs. 8, 9 and 10 show stages immediately succeeding the stage shown in Fig. 7. The threads have become shorter and thicker, the nuclear membrane has entirely disappeared, and the centrosomes have become more widely separated. The amount of segmentation of the thread varies in different cells. In Fig. 11*b* is shown a nucleus in which very little segmentation has taken place, although the thread is considerably thicker than that shown in Fig. 7. The linin threads joining the chromosomes are of extreme delicacy and difficult to discover. Occasionally, however, (Fig. 9) fine fibres may be seen stretching from one chromatin thread to the next. The shortened and thickened chromosomes then arrange themselves into an equatorial plate (Fig. 12). The appearance of the plate, both in side and in pole view, is irregular. The division of the chromosome into chromomeres and their longitudinal division is visible only in very thin sections, which have been stained with iron hæmatoxylin and rather strongly decolorized (Fig. 14). The centrosomes and spindle-fibres of the spermatogonic, mitotic figure are not quite so prominent as those of the spermatocytic divisions. The same is true of the polar radiations. Central spindle-fibres are apparently entirely lacking. After splitting of the chromosomes the halves diverge, in the manner of the two legs of a pair of compasses, the divergence commencing at one end, while at the other end the two halves remain in contact (Fig. 15).

A still later anaphase is shown in Fig. 17. The chromosomes have become massed together, the spindle-fibres are beginning to disappear and the centrosomes are almost lost to sight. The constriction of the cell body, observable to a slight degree at this stage, becomes more marked and a membrane comes to separate the two daughter cells (Fig. 19).

The reconstruction of the nucleus consists of the breaking up of the chromosomes into fine granules, which are connected by linin threads of great delicacy, and in the development of a nuclear membrane (Figs. 19, 20, 21). The change in chemical composition of the chromatin is indicated in sections stained with saffranin and gentian violet by a gradual change in color from red to blue. As the cell body constricts slight thickenings are discoverable on the connective spindle-fibres in the equator (Fig. 18), which, as the constriction proceeds, grow fewer in number and more conspicuous in size until they are finally reduced to a single large swelling, from which radiate the spindle-fibres, by this time grown faint (Fig. 19). At a stage a little later than the one just described I have occasionally seen a small black body wedged in the angle between the daughter cells (Fig. 20). Its appearance is similar to the "Flemmingscher Körper" described by Hoffmann (1898) for *Limax maximus* (see his Figs. 31, 32, 33) and strikingly like that of the rabbit described by von Winiwarter (1900, Figs. 9 and 10).

2. Growth Period.

The anaphase of the last spermatogonic division is decidedly different from that just described. The chromatin threads lie massed together and entangled near the centre of the cell (synapsis). They are surrounded by a clear space bridged over by slender acromatic fibres, which connect the chromatin threads with a narrow layer of cytoplasm lying close to the cell wall. No trace of centrosome or sphere substance (idiozome) is discoverable (Fig. 22). The chromosomes now spread apart, although still connected by strands of linin. They are seen to be for the most part V-shaped. The chromatin granules are rather irregularly distributed, being frequently massed together in lumps (Fig. 24).

In a thin section of a cell at a stage slightly later than this there appeared a minute black dot, surrounded by a vaguely defined area, slightly more dense than the rest of the cytoplasm (Fig. 23). I hesitate to attach importance to this, as it occurred in very few cases.

The threads now elongate, and during this process the granules of which they are made up divide, so that the thread becomes longitudinally split. The granules apparently do not divide simultaneously. Even in the same thread some of them show division, while others remain entire (Fig. 25). The split is to be seen with

the greatest clearness in sections stained with iron hæmatoxylin and strongly decolorized. The chromosomes are very irregularly distributed, only occasionally a part of them, six or seven, may be grouped with reference to a central point. Of the entire number of chromosomes present it is difficult to be certain, owing to the fact that they overlie each other so closely. The number, however, is certainly less than that present in the spermatogonia and not greater than sixteen (Fig. 26). The reduction in the number of chromosomes, therefore, apparently takes place at this stage, and the V-shape so prevalent is due to the approximation of two chromosomes to form a single bivalent one. The place of union is frequently covered by chromatin, but a connection of linin can sometimes be discovered (Fig. 27). This figure also shows the varying angle at which the univalent chromosomes may approach each other. Occasionally they may even form a complete ring.

The threads become more and more attenuated (Fig. 28), and finally by anastomosis are transferred into the nuclear reticulum of the resting spermatocyte (Fig. 30). During the elongation of the chromosomes the chromatin granules divide and redivide (Figs. 23–28), so that they become very numerous, and as the elongation progresses the longitudinal split becomes less easily discoverable, until in the resting cell it can no longer be made out. Cells are sometimes seen in which, just before the formation of the nuclear membrane, the network lies to one side, being connected by slight strands of linin with the surrounding cytoplasm (Fig. 31).

The fact that the chromosomes remain distinct until just before the formation of the nuclear membrane points to a maintenance of their individuality in the resting cell.

The nuclear membrane appears to form as a condensation of achromatic substance, upon which later appear granules staining deep blue with hæmatoxylin (Fig. 29).

A peculiar fact with reference to the last spermatogonic division has struck my attention and I have been unable to explain it very satisfactorily. It will be seen from Fig. 2 that nearly all the cells in the apical portion of follicle (*b*) are in the synapsis stage. It might be supposed from this that sections would be obtained of follicles filled apically with the spindles of the last spermatogonic division. Such a condition, however, I have never found, although I have examined a large number of testes at different seasons of the

year. The karyokinetic figures of the spermatogonia are always scattered and it is impossible to distinguish between the early and late ones.

3. The Maturation Divisions.

In preparing for the first maturation division the meshes of the nuclear network become coarser, the granules more distinct and aggregated into separate threads, joined together by linin (Figs. 32–36). The manner of their origin again lends support to the view concerning their individuality in the resting cell. A still greater condensation of the granules leads to a shortening and thickening of the chromosomes (Figs. 37 and 38), the final result of which is the production of sixteen compact masses of chromatin, still connected by linin threads (Fig. 44). Condensation does not proceed at an equal rate in all the chromosomes of a nucleus. Fig. 45*a* shows a small portion of a nucleus in which lie side by side two chromosomes, in one of which the final dumbbell-shape is almost completed, while in the other the condensation of the chromatin is but little advanced. These sixteen masses are of various forms. Some are dumbbell-shaped, two spheres of chromatin joined by linin; some are crescent-shaped and still others are more or less complete rings (Figs. 39–45). The different forms may occur in the same nucleus, but apparently without constancy in the ratio of relative frequency of occurrence. The dumbbell-shape, straight or slightly curved, is abundant, some cells containing no complete rings (Fig. 41). Other cells contain a comparatively large number of rings or crescents (Figs. 39 and 40).

Two main types may be distinguished among the chromosomes according to their structure and mode of origin—*i.e.*, (1) those in which the bivalent chromosome consists of two univalent chromosomes lying end to end, as in those having the dumbbell-shape, and (2) those in which the univalent chromosomes lie side by side, as in those arising through a ring or narrow V-shape. A form intermediate between these is represented by those having a crescent-shape. The different types and their probable mode of origin are shown in the diagram (Fig. 68*a*, *b*, *c*). It is interesting to note that these types can be distinguished in the synapsis stage (Fig. 27), although they are here not so well marked as in the prophases of the first maturation division.

In cells stained with iron hæmatoxylin, which have been strongly

decolorized, a longitudinal split is evident and likewise a division of the chromosome into chromomeres. If the chromosome is of the second type and seen from above, two of the chromomeres will be seen longitudinally split (Fig. 46; *cf.* Fig. 53). An end view of a chromosome of the first type shows simply a single chromomere longitudinally split (Fig. 59).

Linin connections between the chromosomes are much more evident than in the spermatogonia, and they can be seen to extend from the sides as well as the ends of the chromosomes.

With regard to the origin of the first maturation spindle-fibres it is difficult to be certain, but they appear to arise, at least in part, from within the nucleus. The centrosome is not evident until a rather late prophase (Figs. 39, 40, 43). In many cases it lies within a more densely staining mass of cytoplasm of ill-defined outline applied close to the nucleus (sphere substance, idiozome of Meves) (1898) (Figs. 39*a*, 43). This is not, however, invariably the case, as may be seen from Figs. 40, 39*b*, where the centrosomes lie freely in the cytoplasm. Fig. 38*b* perhaps represents an early stage in the development of the sphere substance. In the two adjacent cells (Fig. 38*a* and 38*c*) are shown rounded bodies of a dull tint lying within clear vacuoles. I met these in but one preparation and am unable satisfactorily to interpret them. The division of the centrosome and the formation of the spindle is shown in Figs. 46, 47, 48, 52. The centrosomes and spindle-fibres, as well as the polar radiations, are more prominent than in the spermatogonic spindles. During this time the sphere substance disappears.

In the equatorial plate the chromosomes become arranged with the longitudinal split parallel to the axis of the spindle in the case of chromosomes of the first type, but at right angles to it or nearly so in the case of chromosomes of the second type (Figs. 49, 50 and 53). In Figs. 55 and 56 are represented pole views of both types of chromosomes. It may be gathered from these, as well as from the figures of the prophases, that chromosomes of the second type are not nearly so numerous as those of the first nor so numerous as those of the intermediate type.

From what has been said with regard to the origin of the chromosomes, it will be seen that in the metaphase the bivalent chromosomes are separated into their univalent components, and consequently the *first division is one of reduction.*

A well-marked mid-body is visible in the late anaphase (Fig. 61*a*). The interzonal fibres are sharply constricted and oftentimes the nuclei completely separated before a cell wall makes its appearance. In stages like this a noticeable bending of the fibres is often observed. This is slightly evident in Fig. 61*a*.

Apparently the plane of the second spermatocytic division is to be at right angles to the first, if Figs. 61*a* and 61*b* are interpreted as early stages in the formation of the equatorial plate of the second spermatocyte.

The equatorial plate of the second spermatocytic division is shown in lateral view in Fig. 62. The length of the chromosomes is less than that of the chromosomes of the first spermatocytic division. The question as to whether the second division is actually equational is difficult to decide. The chromosomes of the first maturation figure, consisting of a double row of four granules, are separated by karyokinesis into halves, and each half contains a double row of two granules (Fig. 58). It thus has the appearance, although only the appearance, of a true tetrad. It will be seen that some of these daughter chromosomes have a length equal to their width, whereas in others the length is slightly greater than the width. If we turn to the fully-formed spindle of the second division (Figs. 62, 63) we find similar phenomena. It might be argued from these appearances that the second division is also reducing. In view, however, of the weight of evidence in favor of both methods of division (equation and reduction) being necessary to the maturation of the sexual cells among the Arthropods, I hesitate to accept this interpretation without further corroborative evidence. When the length of the chromosomes is equal to their breadth, it is obviously as impossible to decide here concerning the plane of division as in the case of the true tetrads of the Copepods, *Canthocamptus*, *Heterocope* or *Diaptomus*. If the length is greater, as in the anaphase, the appearance might be referred to the elongation of the mother chromosome (Figs. 49, 51, 53), some of the daughter chromosomes not having recovered from the stretching apart of the chromatin in the metaphase. The apparently greater length of some of the chromosomes in the spindle of the second division (Fig. 63) may be explained by the assumption that some of the chromosomes commence to divide earlier than others, and consequently become elongated, an assumption which is not without parallel in the first spermatocytic and especially in the sperma-

togonic divisions (Fig. 15). In Fig. 61, a stage intermediate between 58 and 62, some of the chromosomes likewise appear of greater length than others. It might be supposed that the longer ones represent the side view, the shorter ones the end view, of the chromosomes. This need not, however, necessarily be the case, for the chromosomes vary amongst themselves in size (Fig. 58 and previous figures). It is possible, too, that in some cases the chromosomes are seen slightly foreshortened and that their true dimensions do not appear in the figure. I feel it, therefore, impossible to ascertain with the desired degree of certainty the plane of the second spermatocytic division.

In the late anaphase (Fig. 66) the chromosomes are more or less indistinguishably massed together. On each of the interzonal fibres in the equator is a minute swelling. These become reduced in number (Fig. 67).[1]

4. Metamorphosis of the Spermatids.

The chromosomes spread apart, a nuclear membrane is developed and the daughter cells become the spermatids. The gradual conversion of the chromosomes into a fine reticulum is illustrated in Figs. 69 and 70.

The nucleus now commences to elongate at one end (Fig. 72), and this continues until the entire nucleus is transformed into a shape somewhat like that of a narrow flask (Fig. 74). The nuclear network is extremely delicate and takes the iron hæmatoxylin stain more faintly than previously. In cross section (Fig. 74*b*) numerous fine dots appear interspersed with clear areas (vacuoles). This vacuolated appearance is sometimes evident at an earlier stage (Fig. 71).

During the transformation of the nuclei the cell boundaries have entirely disappeared and the nuclei lie in a common mass of cytoplasm. Several of them become associated together, and their extremities, elongated into slender threads, are surrounded by a clear, homogeneous, well-defined area of cytoplasm, while the more or less contorted bodies of the nuclei still lie in an undefined mass of cytoplasm (Fig. 77*a*).

A cytoplasmic thread of extreme delicacy can be traced from the

[1] During the examination of the foregoing stages I have seen nothing similar to the accessory chromosome (chromatin nucleolus) of insects, as described by Montgomery (1898) and Paulmier (1899).

slender extremity of each nucleus for some distance into the clear, homogeneous area (Fig. 77*a*). At this stage also there can be clearly seen in the undefined mass of cytoplasm a bundle of fibres, which run in between the nuclei, but which cannot be seen to have any connection with them. I have a preparation of a stage, earlier than that just described, stained with hæmatoxylin and Bordeaux red, in which these striations appear, near the margin of the follicle (Fig. 73). So early a development of the fibres is rather unusual. The fibres are here apparently incomplete and not massed together as they later are. On account of their indistinctness it is difficult to say whether or not they are independent of the nuclei. At first sight it might appear as if they were continuous, but it is impossible to state definitely that this is so because of the impracticability of tracing a single fibre for any great distance.

The further changes in the nuclei consist in their gradual elongation into filaments, in which the network has entirely disappeared and which have acquired the power to take a vivid and homogeneous stain. Their free ends, at first divergent, gradually approach each other and finally come to lie close together (Figs. 77–79, 85 and 86). In regions of the follicle where the cells are closely crowded together the nucleus is often seen to be bent or coiled upon itself (Fig. 83).

There is at first a small quantity of cytoplasm around the nuclei, but as they increase in length this disappears. The cytoplasmic fibres also increase in length at the expense of the surrounding cytoplasm. Their length, indeed, becomes truly marvelous, many times exceeding that of the nuclei. They crowd in between the follicle cells (Fig. 2) and in cross sections of the follicle can be seen in great numbers around the margin. From the anterior end of the bundle is developed a slender flagellum (Figs. 85, 86). The entire bundle has the appearance at first sight of a single spermatozoon, and such I thought it before having studied its development.

The term "spermatophore" has been applied by Gilson to the bundle. This term, however, has been used by Grobben and others to designate an envelope secreted by the cells of the vas deferens (in the Decapods) and surrounding a mass of spermatozoa. It does not, therefore, seem applicable to the bundle of spermatozoa found in the Oniscidæ. Ballowitz applies the term "spermozeugma" to a large bundle of double spermatozoa found in the vas deferens

of the Dytiscid, *Colymbetes striatus*. These adhere together after having reached maturity. Their structure and mode of origin is, therefore, not the same as that of the bundles of Oniscus. The term "compound spermatozoon" has been suggested to me, but the word spermatozoon might carry with it certain implications with regard to behavior in fertilization. I prefer, therefore, to use the term sperm colony, at least until a better one offers itself. Gilson uses this term also, although not so generally as the word "spermatophore."

The number of nuclei entering a colony varies within rather wide limits. I have counted as few as six and also as many as fourteen. In cross sections stained with saffranin and malachite green, they are seen as red bodies surrounding a central mass of green dots, the sections of the cytoplasmic fibrils (Fig. 80). The red dots diminish in size toward the anterior end of the bundle, and at one point can be seen merging directly into delicate green threads (Fig. 80*b*). At the extreme anterior end of the bundle the delicate green threads alone will be cut (Fig. 80*a*). It might be supposed that the bundle of cytoplasmic fibres previously described are the tails of the spermatozoa. If they are really the tails of the spermatozoa, one would expect to find them at some place connected with the nuclei, or with the delicate fibres which can be demonstrated to be continuous with the nuclei. A comparison of sections obtained at different levels seems to leave but two alternatives: either the long bundle of cytoplasmic fibres stops abruptly before the anterior end of the colony is reached, or the connection is of so tenuous a character as to escape observation. In structures of such minuteness the latter might easily be the case.

A point bearing on this matter, and therefore of interest to determine, is the number of cytoplasmic fibres as compared with the number of nuclei. Attempts to determine this might be made in two ways. The mature sperm colonies taken from the vas and teased apart might be examined and an attempt made to count the fibres at the frayed end of the bundle, or one might try to count the number as seen in cross sections. By either method it is difficult to be sure of an accurate count, for in the frayed ends of colonies one or more of the fibres may adhere together. In cross sections the fibres appear as minute dots, as a rule, closely crowded together. Occasionally they may be more loosely distributed. Fig. 81*b* represents a cell of this sort in which the number of cyto-

plasmic fibres equals that of the nuclei. I cannot be certain that this is invariably the case. With the iron hæmatoxylin stain the bundle of cytoplasmic fibres stains deeply, like the nucleus, and it is therefore impossible to distinguish between them in cross section where both appear. The delicate fibril previously mentioned, which joins the nucleus, stains faintly and can therefore be distinguished from the nucleus. In Figs. 81 and 82 cross sections of sperm colonies at slightly different stages of development, colored with this stain, are compared. In both images may be seen similar to that of Fig. 80—*i. e.*, a circle of dots merging into faintly staining fibres. Sometimes the latter have a granular or beaded structure (Fig. 81*a*). These are sections near the anterior end of the colony, and here again the central circle of dots, representing the posterior cytoplasmic fibres, is lacking. Fig. 82*b* represents a section which I interpret as having been cut slightly posterior to Fig. 82*a*. The tail fibres here begin to appear.

A comparison of the two stages illustrates the gradual dwindling of the cytoplasm which surrounds the bundle. It will be remembered that shortly after the complete reconstruction of the spermatid nucleus, cell boundaries disappear and the nuclei lie in a common plasma. When, however, the nuclei come to be associated in groups, the cytoplasm again becomes sharply defined and in cross sections an appearance like that of separate cells is obtained (Fig. 80). The cytoplasm in the anterior region becomes comparatively homogeneous and the nuclei often lie in a central clear space (Fig. 81*a*). More posteriorly it breaks up and assumes a granular appearance (Fig. 81*c*), while still farther back the fibrillar bundles lie isolated, with vague remnants of cytoplasm between them (Fig. 81). In Fig. 82 the diameter of the colony is less and the cytoplasm surrounding the fibres decidedly less extensive.

The sperm colonies when mature, or nearly so, are forced into the vas deferens, probably by contractions of the muscle layer of the follicle. In the vas they are surrounded by a fluid secreted by the large cells which form its lining, and which causes them readily to adhere to needles or forceps. The mature colony has the appearance shown in Figs. 85, 86 and 87. I have not heen able to isolate a single colony entire, for in teasing the long fibres are almost invariably torn. I have been able to trace them for a considerable distance, however, and can state that they are exceedingly long. The filamentous nuclei are invariably partially frayed from the

sheath and often entirely torn from it, lying twisted and contorted at some distance from the sheath. According to Hermann, 1883 (*2*), the spermatozoa of the Isopods retain their immobility in the oviduct of the female. The function of the extraordinarily long fibres, if the spermatozoa remain motionless, is to me a matter of great perplexity. It becomes still more puzzling if, as my preparations seem to indicate, there is no direct connection between them and the nuclei. Their function and their true relation to the nuclei might possibly be elucidated by a study of their behavior in fertilization, a study in which I hope to engage at some future time.

5. The Nucleolus.

In the resting spermatogonia the nucleolus is present as a rounded or oval body, staining pink with the eosin of the Biondi-Ehrlich stain and red with saffranin. When the mitotic figure is fully formed it is, as a rule, no longer visible, nor is it seen in the prophase immediately preceding. The newly constructed daughter nuclei likewise show no trace of it (Figs. 10, 14, 20). Possibly it may consist of metabolic products developed in the resting cell and quickly dissolving during or before mitosis. In the synapsis stage, subsequent to the last division of the spermatogonia, the nucleolus is, however, clearly visible, lying to one side of the tangled mass of chromatin threads.

In the very earliest synapsis of which I have sections it is not discernible (Fig. 22), but as the threads elongate and separate it becomes evident. It continues to be present throughout the synapsis and is finally enclosed within the nucleus of the resting spermatocyte by the development of the nuclear membrane (Figs. 23, 26, 28, 29 and 30). Throughout the prophases of the first spermatocyte it is still to be seen within the nucleus (Figs. 32, 33 and 43*a*), and after dissolution of the nuclear membrane and formation of the mitotic figure it is cast off to one side of the spindle, where it persists for some time (Figs. 47, 51, 52, 55, 61, 65–67 and 69*b*). With saffranin and malachite green the nucleolus is very evident, coloring bright red, while the chromatin of the resting cell is purple. With iron hæmatoxylin it is not so readily distinguished, but with the Biondi-Ehrlich stain it can be seen as a pink body lying to one side of the spindle.

6. Summary.

The main results of this study may now be briefly summarized as follows:

(1) The spermatogonic chromosomes are joined together in pairs in the synapsis to form sixteen bivalent chromosomes. They may be joined (*a*) in an approximately straight line, (*b*) to form a more or less narrow V, or (*c*) into a more or less complete ring (Figs. 26, 27).

(2) A longitudinal splitting of the chromatin threads takes place at this stage (Figs. 25*a*, *b*, *c*).

(3) The distinctness maintained by the chromosomes up to the formation of the nuclear network of the resting spermatocyte, and the manner of origin of the spermatocytic chromosomes from it, lends support to the theory of their individuality in the resting nucleus (Figs. 28 and 32).

(4) In the structure and mode of origin of the bivalent spermatocytic chromosomes two main types may be distinguished: (*a*) The component chromosomes lie end to end, or (*b*) they lie side by side (Figs. 68*a*, *b*, *c*).

(5) Inasmuch as univalent chromosomes are separated, the first maturation division is reductional (Figs. 48–59).

(6) Sphere substance (idiozome) is not observable, except for a short time during the prophases of the first spermatocyte (Figs. 39 and 43).

(7) The nucleolus of the spermatogonia disappears shortly after dissolution of the nuclear membrane, while that of the spermatocytes, first discovered in the synapsis, persists throughout the divisions (Figs. 8–10, 47, 26, 29, 33, 47, 48, 51, 52, 55, 58, 60*a*, 61, 65–67, 69).

(8) The spermatids become associated in groups to form colonies of nuclei lying in a common plasma (Figs. 73–75).

(9) Within the latter arise bundles of fibres of great length, whose connection with the nuclei, if actual, is very slight and occurs very late, as well as single fibres of greater delicacy which are continuous with the nuclei (Figs. 76–83).

(10) The mature sperm colony consists of a variable number of filamentous nuclei contained, together with the bundle of cytoplasmic fibres, in a tenuous sheath which is flagellate at its anterior extremity (Figs. 84–86).

III. Critical Review of the Literature on Crustacean Spermatogenesis since 1878.

1. Spermatozoa.

a. Review.

Decapoda.

1878. Grobben in his valuable work investigates principally the form of the Decapod spermatozoa and their transformations from the immature to the mature state, as well as the nature of the case (spermatophore) in which they are enclosed. With regard to the spermatozoon of Astacus fluviatilis, he states that the head develops from a structure arising near the nucleus, while the nucleus itself disintegrates. He gives also a review of the literature on Crustacean spermatozoa up to that time, which therefore need not be repeated here.

1883 (1). Herrmann describes the spermatozoa of the Podophthalmia, chiefly the Macrura and Brachyura. The study of the development, he says, shows a series of transitory forms which enable us to seize clearly the bonds of relationship existing between the different adult forms. The transitional forms of some resemble the complete forms of others.

1884. Nussbaum (Astacus fluviatilis) considers the change of the spermatid into the spermatozoon. He traces the gradual condensation and transformation of the nucleus from spermatid to spermatozoon, and the transformation of a large body lying in the cytoplasm into the peculiar "kopfkappe" of the mature spermatozoon (see his Figs. 53–68). He regards the nucleus as the head of the spermatozoon.

1885. Sabatier published a short article on the spermatogenesis of the Decapod Crustacea, principally Astacus.

1886. Gilson describes the spermatozoa of a considerable number of Decapod species, among others Astacus fluviatilis. The structure of the spermatozoon of the latter he delineates more fully than either of his predecessors. The nucleus he shows to be present and saucer-like in shape. It is covered by a layer of protoplasm which is extended laterally into pseudopodic processes. From the centre of the protoplasmic layer sometimes arises a protuberance, to which he gives the name "globule achromatique." The nucleus surmounts a bladder-like vesicle often perforated at the opposite pole. Into this from the centre of the concavity of the nucleus projects what he calls "la tigelle."

1895. Auerbach compares the spermatozoon of Astacus fluviatilis with those of other Crustacea, Insects and Vertebrates, with a view to discovering homologies of head, apex, middle-piece and tail. The cyanophilous, saucer-shaped nucleus corresponds to the head of more highly developed spermatozoa, its pole therefore to the anterior end of a flagellate spermatozoon and the surrounding protoplasm to the sheath of the head. The "globule achromatique" of Gilson is the anlage of the apex. The "tigelle" of Gilson, which Auerbach found to be erythrophilous, he regards as the anlage of the middle-piece. In the genera Pagurus, Eupagurus, Clibanarius and Ethusa the "tigelle" is prolonged into what Auerbach regards as a rudimentary tail. The bladder-like vesicle is perhaps a kind of "Schwanzkappe," possibly comparable with the sheath sometimes surrounding the place of origin of the tail in immature vertebrate spermatozoa. The extremity regarded by Grobben as the head would, according to Auerbach's interpretation, be the tail end. For a more detailed account of the Decapod spermatozoa, of which that of Astacus may be taken as a type, the reader is referred to the works cited above.

Stomatopoda, Schizopoda, Amphipoda.

1885. Gilson, in his excellent and very comprehensive work, describes also the spermatozoa of the Stomatopod Squilla, the Schizopod Mysis and the Amphipod Gammarus. The whip-like spermatozoon of Mysis is strikingly similar in shape to that of the Isopods. That of Gammarus is flagellate and that of Squilla vesicular.

Isopoda.

1883. Herrmann studied among the Isopoda, Ligea, Idotea and Sphæroma. His description is unaccompanied by figures and is difficult to comprehend. The spermatic filaments, he says, are united in numbers varying from eighty to one hundred. The bundles are found lying amongst the cells which line the walls of the tube. He did not find isolated spermatozoa, except in the oviduct of the female, where they retain their habitual form and immobility. The large cell of the vas deferens he considers as homologues of ovarian cells and calls them "ovules males."

1884–1886. Gilson (Oniscus asellus). Groups of six spermatids

("spermatoblaste") were observed surrounding a protoplasmic stem and their origin referred to the small cells in the apical portion of the cæcum. The structure of the nuclei and the changes in them and in the surrounding protoplasm, by which the mass is converted into the mature "spermatophore," are described at some length and illustrated with numerous figures. The name "spermatophore" is applied for the following reasons: "Les cellules spermatozoides sont donc contenus dans un étui resistant derivant de la differentation du protoplasm, c'est-a-dire dans une production particuliere, on pourrait donc appliquer aux faisceaux la denomination de spermatophore." The name "plasmodium pariétal" is applied to the follicle cells and the surrounding protoplasm, and to it is ascribed the function of taking part in the formation of the tails, thus reinforcing the insufficiency of the protoplasm of the germ cells. The tails of the spermatozoa are thus thought to arise in the plasma and to attach themselves to the nuclei "vers le haut." The exact level is not determined. The form of the spermatozoa is compared to that of a whip, the long tail representing the handle and the nucleus the lash. This would seem to indicate that the tail is conceived as being attached to the nucleus at its upper extremity. The entire bundle is said to measure 0.15$\frac{5}{6}$ mm.

The sheath (étui) enclosing the spermatozoa is most evident at the anterior end. The apparent absence of protoplasm around the filamentous nuclei is explained as perhaps due not to degeneration or absorption of the protoplasm, but to a condensation and fusion with the nucleus, perhaps applying itself so closely to the filament that an effect of refraction communicates to it the same coloration This hypothesis is based on results obtained by treating the flagellæ with nuclear solvents. When submitted to the action of potassium carbonate in concentrated solution or strong hydrochloric acid for several days the filaments become scalariform; a skeleton formed of little chambers is seen which communicate with each other, and which were previously filled with the nuclear substance. The characteristic frayed appearance of the bundles is thought to be due to artificial rupture.

The nuclear flagellæ are said to grow considerably after having attained their distinctive form. From the figures given to show this (Figs. 329 and 330, Pl. VIII), it seems probable that this appearance may be due to a portion of the filaments having been broken off by teasing.

The large cells lining the vas deferens are described and also the smaller cells between them. The latter are believed to arise from the larger ones by segmentation. The function of the large cells is said to be the secretion of the fluid which bathes the spermatozoa. The nucleus of these cells is figured as a network of great regularity.

The mature colony of Asellus, as figured by Gilson in Vol. 2 of La Cellule, Pl. X, Figs. 385–395, agrees with that of Oniscus in general appearance. The spermatozoa in the bundle, however, are more numerous and much less compactly bound together. Associated with them in their development is a large cell ("noyau femelle"). The tail is shown to be distinctly continuous with the nucleus. The granular mass surrounding the nucleus at its free end is said to consist of caryoplasm and the remains of the nuclear membrane. Its formation is shown in Figs. 387–393.

A few figures are also given of Idotea.

1886. Wielowieyski, in a short paragraph concerning Asellus, states his opinion that the "noyau femelle" of Gilson is an artificial product, caused by the confluence of the protoplasmic mass with one of the large cells on the margin of the testicle.

Cirrepedia.

1886. Gilson figures the spermatozoa of Lepas anatifera and Balanas perforatus. They are flagellate, the nucleus a slender thread occupying the anterior end.

1894. Ballowitz, K., studied Balanas improvisus Darw. and Lepas anatifera L. He makes the astonishing statement that the head is demonstrable as a distinct structure neither by its form nor by its staining reaction. He mentions the work of Nussbaum (1890) on a Californian Cirrepede (Pollicipes polymerus) in which the head is described.

Copepoda.

1895. Steuer gives a figure to show the spermatozoa of the marine Copepod, Sapphirina gemma. They are flagellate, shaped somewhat like a javelin. He mentions the spermatozoa of the Calanidæ as being of spherical shape.

Ostracoda.

1886. Stuhlmann. The spermatozoa of the Cypridæ are described as having at first the shape of a ribbon, through the length

of which the nucleus runs as a thread. They are stated to increase in size through the assimilation of a secretion of the vas deferens. They then become spirally twisted while in a certain limited section of the vas deferens, presumably by a motion of their own. This is said to be caused by a fibre running spirally the length of the spermatozoon. The mature spermatozoon has the spirally twisted structure of a rope of tow. It contains a twisted central fibre, not visible externally, and the entire structure is surrounded by a hyaline sheath. The spermatozoa are nearly motionless while in the body of the male, but become extremely active in the receptaculum seminis of the female. This is said to be due to the loss of the hyaline sheath. The curious fact is noted that the spermatozoa coming from the right side of the animal are twisted to the left and vice versa.

1889. Müller discovered in the spermatid of Ostracoda one or two "Nebenkerne." These form a "Schwanzstuck" which grows very long and is of complicated structure. Through the middle of the tail runs the central fibre, at or near one end of which the nucleus is located. The spiral twisting is referred to the contraction of the middle one of the three threads which surround the central fibre. He does not agree with the opinion of Stuhlmann concerning the inhibitive function of the sheath while in the body of the male.

Phyllopoda.

1885. Zacharias describes the results of his observations and experiments on the spermatozoa of the Phyllopod, Polyphemus, which he shows to be capable of amœboid movements.

b. Commentary.

The Crustacea as a class show an astonishing variety in the form of the male reproductive elements. Knowledge of their intimate structure is of course at present too incomplete to enable us to discuss at any great length the homologies existing between them. But a rough classification of them according to their external appearance would place the bell-shaped or vesicular form characteristic of the Decapods in one group and the form found in the Isopods, Gammarus, Mysis and Balanus, with more or less elongated nucleus and tail of varying length, in another. The extremely peculiar form of the spermatozoon of the Ostracoda might perhaps be referred to the

latter group. It is possible, and I advance it simply as a tentative hypothesis needing corroboration, that these strikingly dissimilar forms have arisen from a primitive one, simple and amœboid in character like that of Polyphemus.

The ingenious series of homologies drawn by Auerbach between the head, tail, apex and middle-piece of the spermatozoa of Vertebrates and Insects and similar structures in Astacus appears plausible. Since, however, the location of the centrosome and the sphere substance remains undetermined, the homologies cannot be said to be in all respects established. A more detailed and thorough examination of the spermatozoa of the Crustacea, especially of their behavior in fertilization, might extend these homologies. If the spermatozoon of Oniscus be compared with the type most frequently occurring in animals, the part immediately adjacent to the nucleus, the delicate fibril shown in Figs. 77*a*, 79, corresponds in location to the middle-piece. Whether this is in reality the habitation of the centrosome might be discovered through a study of its fate during fertilization. My observations on the spermatogenesis throw no light on the question.

The Isopods are unique among the Crustacea in the formation of colonies of spermatozoa of a nature so close that they appear as units. Concerning their origin in Oniscus, I can confirm M. Gilson's statement that the formation of the bundle takes place in a plasmodium, cell boundaries being for a time entirely absent, and with the main outlines of his account of the changes taking place in the development of the spermatids into the mature colony I am thoroughly in accord.

The number of nuclei entering into a bundle, according to my observations, is not invariably six, but may vary within considerable limits. The number of cytoplasmic fibres is assumed by M. Gilson to be equal to the nuclei, but in his Fig. 328, Pl. VIII, they are shown to be more numerous. As has been already said, I have been unable to convince myself of a direct continuity between these fibres and the nuclei. In his Fig. 320 (an immature spermatophore) the cytoplasmic fibres may be traced directly to the nuclei. I have, however, not been able to obtain images of equal clearness from my preparations. Nor have I obtained anything at all similar to the rings or vacuoles, shown in Gilson's Figs. 328, 329 and 330, near the anterior end of the bundle. In Sphæroma serratum, Gilson states, the continuance of head and tail is very evident, forming an

open and regular ring. The close relationship of nucleus and cytoplasmic fibre in Oniscus is shown only in Fig. 320. In Figs. 323 and 326 they are represented as discontinuous. In Fig. 334 (Asellus) the fibres are pictured as arising independently of the nuclei, although it is shown in later figures that they eventually become attached. If the follicle nuclei and the surrounding protoplasm take part in the formation of the tails, it is only, in my opinion, in so far as they become converted into the substance of the germ cells.

In attempting to reconcile the fact of the direct continuity of head and tail, shown by Gilson so clearly in Asellus and stated by him to be present in Sphæroma, with the lack of demonstrable connection in Oniscus, it occurred to me that the condition in Oniscus might represent a different phase in the evolution of the Isopod spermatozoon. Either the connection, at one time evident, between the nucleus and the unusually long tail may have grown so slight as to be no longer recognizable, or, if the spermatozoon of Oniscus for any reason is to be looked upon as the more primitive form, it may be that the connection, which will later in the course of evolution become more marked, is as yet but little developed. Although in the present state of our knowledge both alternatives may perhaps be considered open, the former seems to me far more plausible, for not only are the land Isopods in other structural peculiarities to be regarded as more specialized than Asellus, but the sperm colony itself in Asellus is less compact and less completely developed as a unit. The obscurity of this point serves to emphasize the desirability of further study of the Crustacean spermatozoa and the establishment of accurate homologies between them.

The "noyau femelle" of Asellus is, in my opinion, to be regarded as homologous with the follicle cells of Oniscus. I am inclined to doubt the correctness of M. Gilson's conclusions as to the origin of the small cells of the vas deferens of Oniscus from the larger ones by segmentation, and, although I have not devoted much time to the elucidation of the point, I think it more probable that the reverse is true, for I have seen the small cells segmenting, but never the large ones.

2. The Earlier Stages in the Development of the Germ Cells in Crustacea, with Especial Reference to the Problem of Reduction.

a. Review.

Decapoda.

1878. Grobben gives almost no figures of the earlier stages and does not consider the subject in detail.

1884. Nussbaum (Astacus fluviatilis) does not distinguish between spermatogonia and spermatocytes. Five figures of mitoses are given in which the chromosomes are shown to be spherical at the beginning of the metaphase, but they soon elongate to a rod-like shape.

1885. Carnoy studied among the Decapods, Astacus fluviatilis, Crangon vulgaris and several species of Brachyura and Anomura. In no case are more than thirteen figures given. It is impossible to determine in every case the generation to which the cells belong. The mode of origin of the chromosomes is not fully traced, and it is impossible to determine with accuracy, therefore, anything with regard to the question of reduction. In the case of Astacus, as far as can be judged from the figures given (Figs. 246*a*, *b*, *c*, *d*, *e* and *f*), the division is transverse. The mitosis figured occurred in August, and, according to vom Rath, it is from this month until December that the final divisions of the spermatogonia and those of the spermatocytes take place. A transverse spermatogonic division is improbable. The chromosomes are shown to arise, however, through the shortening and thickening of rods, resulting from the breaking up of the nuclear network. The transverse division, if it be such, is therefore probably that of the first spermatocyte. The same is perhaps true of Crangon cataphractus (Figs. 247 and 248). Of peculiar interest is the constitution of the chromosomes of Crangon cataphractus, as shown in Figs. 249*a*, *b*, *c*, *d*, Pl. VII. According to these a chromosome in longitudinal view consists of a double row of from three to five granules. A reconstruction of the chromosome from these figures leads to the conception of a rod split longitudinally several times.

Cytoplasmic Structures.—A dense mass, lying within the cytoplasm during the prophases and migrating to the poles of the spindle as it is formed, is shown for Crangon. No centrosome is figured as lying within this mass, to which the name "Nebenkern" is given. The same name is applied to a body lying in the cyto-

plasm in Astacus. This body, however, seems not to be affected by mitosis, but lies passively to one side. In the vicinity of the poles are, however, numerous granules ("corpuscles polaires") (Fig. 246*f*, Pl. VII). The "Nebenkern" of Crangon, according to the description, behaves like the substance designated idiozome by Meves. The "corpuscles polaires" of Astacus may be of a similar nature. For the other forms studied no bodies of any kind lying in the cytoplasm are shown. The substance seems to be unusually prominent in Crangon and Astacus. The cells of both are of large size.

1891. vom Rath settled the question of amitotic division of the germ cells of Astacus in the negative. He states that a minority of the spermatogonia undergo no change at first, but give rise by mitosis to new spermatogonia after the discharge of the ripe spermatozoa. He mentions a case of regeneration of an entire follicle from a single spermatogonium. With the first appearance of the spermatids the follicle cells ("Randkerne") commence to grow in size and divide amitotically. The direct division apparently takes place by a sharp breaking apart of the portions of the nuclei, resembling a slicing. Degeneration of the nuclei follows. At the point of transition between follicle and duct there is often an extraordinary growth of cells by amitosis. The results of his research are interpreted by vom Rath to mean that two kinds of cells have arisen from indifferent epithelium, one dividing mitotically, the other amitotically.

Isopoda.

1884. Gilson states that it is only at certain seasons of the year that the spermatogenesis of these animals can be studied with profit. In the case of Oniscus asellus, from July to November is the most favorable season for obtaining preparation of what he calls the first stage ("premiere etape"). In the case of Asellus aquaticus it is later—about the month of February.

Oniscus asellus.—The cells filling the apical end of the cæca (spermatogonia) are mentioned, and the opinion is expressed that they constitute a reserve mass destined to replace by proliferation the elements organized in the lower part of the tube and later evacuated. Karyokinesis in these cells (spermatogonia) was observed but once, and the stages intervening between them and the spermatocytes were not discovered.

The condition of the lower part of the tube is thus described: "Il y aurait dans les cæcums testiculaires des Oniscus une sort de plasmodium contenant une grande nombre de noyaux et entourant une masse centrale formée d'éléments spermatiques en formation. Ce fait est si étrange qu'on n'ose à peine l'accepter." The amitotic division of nuclei occupying the lower portion of the follicles and referred by Gilson to the germ cells is probably that of follicle cells, for they are described as occupying the periphery of the tube in its median portion.

1885. Carnoy makes the following statement concerning the Isopods (pp. 222, 223): "Chez l'Oniscus asellus, au moment de la plus grande activité cellulaire preludant à la formation des spermatozoïdes, on ne rencontre pour ainsi dire que des noyaux en voie d'étranglement ou de division acinétique. Les figures caryocinétiques y font le plus souvent défaut. Depuis trois ans nous n'en avons rencontré que deux, une couronne equatoriale et une couronne polaire qui sont reproduite dans la Pl. VI, Fig. 227; et cependant nos observations ont été nombreuses et pratiquées à toutes les époques de l'année.

"Nous avons constaté les mêmes phénomènes sur plusieurs animaux du même groupe, sur les Idotea en particulier. La division directe est très fréquente chez ces derniers, et s'y fait normalement. Nous n'y avons point remarque de caryocinêse; mais nous devons ajouter que nos observations sur ces Crustacés bien que fait serieusement ont été beaucoup moins nombreuses que sur Oniscus. Chose remarquable, chez les Idotea la multinuclearité des grandes cellules qui vont se transformer en autant de faisceaux de spermatozoïdes est due exclusivement à la segmentation du noyau primitive. Ces faits sont d'autant plus singuliers que dans un genre voisin, le genre Armadillo, les figures caryocinétiques sont frequents; tandis que les cas de division directe y sont beaucoup plus rares." I have examined testes of Armadillo and also of Porcellio and find that they do not differ greatly from Oniscus as to the manner and frequency of the divisions.

Copepoda.

1890, 1892. The work of Häcker on the eggs of Cyclops has been corrected by the later research of Rückert and need not, therefore, be mentioned here.

1892. Ishikawa gives a figure of the testis of a Copepod cut

longitudinally, showing it to be divided into regions called by him formative, growing and ripening zones. The formative region corresponds in Oniscus to the reserve groups of spermatogonia, the growing region to the apical part of the follicle and the ripening zone to the basal part of the follicle. Ishikawa's conclusions concerning reduction have not been substantiated by recent research.

1894. Rückert. This well-known paper concerns the ovogenesis of the Copepods, *Cyclops strenuus*, *Heterocope* and *Diaptomus*.

In *Cyclops* the number of chromosomes is 22–24. The germinal vesicle shows double threads of chromatin, a longitudinal split having occurred at an early period. At the beginning of maturation these contract to double rods, whose number is the reduced one and which have, moreover, become transversely split. As the spindle is formed the chromosomes come to lie in the equator, with the longitudinal split at right angles to the axis of the spindle. The first division is thus equational. In the second division the chromosomes are separated along the transverse split, and this division is therefore reducing.

In *Heterocope* and *Diaptomus* open rings are formed which, through condensation, become the tetrads. The plane of the first division is not so easily determined for these Copepods. In the opinion of Rückert the first maturation division of Diaptomus is equational.

1895. Häcker studied the ovogenesis of the Copepod, *Canthocamptus*. The reduced number of chromosomes is twelve. There are apparently two divisions of the ovogonia. The last division is followed (1) by a transverse breaking apart of a doubly split thread and a shortening and thickening of the segments so that twelve double rods are produced. Some of these are transversely split. Or (2) the last division of the ovogonia is followed by a condensation and longitudinal division of the thread as a whole and a subsequent breaking apart of the thread into twelve double rods. These become transversely split and form chromosomes corresponding to the tetrads of the first mode. In either mode the changes follow immediately upon the last division of the ovogonium, and no true reticulum is formed in the germinal vesicle. Since the width of the chromosomes is equal to their length, it is impossible to settle the question as to the order in which the longitudinal and transverse divisions occur.

1895. vom Rath describes the ovogenesis of marine Copepods

mentioned by him in his earlier works on *Gryllotalpa* and *Salamandra*. He studied the genera *Euchæta, Eucalanus, Anomalocera* and *Pleuromma*. He calls attention to the differences that may exist between the ovogenesis of different species of marine Copepods and between the ovogenesis and the spermatogenesis of the same species. His conclusions on the subject of reduction agree substantially with those of Rückert. Particularly in the case of *Euchæta marina* and *Eucalanus attenuatus* is the aspect of the first maturation figure similar to that of Cyclops. Here, too, the division seems to be equational.

Ostracoda.

1898. Woltereck describes a well-marked synapsis zone in the ovary of a parthenogenetic Cyprid. He rejects, as not applying to the object which he studied, the theories of Moore, Brauer and Häcker concerning the relation of the synapsis to the last ovogonic division and to the processes of reduction and maturation. "Von 'Reduktion,'" he says, "ist nicht die geringste Andeutung vorhanden, von der Reifungstheilung sind die Eier noch durch eine lange Phase getrennt, in der das Chromatin kaum sichtbar ist and gegen die Auffassung als Dispirem die excentrisch Zusammenballe bei deutlich vorhandenem Nucleolus, sowie das Vorhandensein aller Uebergänge aus einem lockeren, hellen Fadenknauel in die Synapsis und aus ihr in die segmentirten Chromosome."

Phyllopoda.

1892. Brauer thus summarizes his results on the ovogenesis of Branchipus: "Die Beobachtungen, welche ich bei Branchipus gewonnen habe, zeigen nun folgendes Bild:

"1. Keimbläschen: durch Quertheilung entstehen 6 Schleifen; eine neue Quertheilung erhöht ihre Zahl auf 12. Dann folgt eine doppelte Langspaltung. Resultat: 12 viertheilige Chromosomen bil den die Aquatorialplatte der ersten Richtungsspindle" (p. 53). In describing the Figs. 8 and 9, Taf. I, upon which he bases this conclusion, he says: "Ich will gern zugeben, dass diese Beobachtung schwierig sind und eine Taüschung möglich ist, doch muss ich vorheben, dass ich kein Bild gesehen haben, welches eine Vermehrung der 12 Faden durch eine Quertheilung auf 24 zweitheilige auch nur andeutete und spatere Verklebung von je zweitheilige zeigte. Solche Bilder, welche ganz ähnlich aussehen

müssten wie das in Fig. 1 dargestellte, waren mir, glaube ich, nich entgangen."

1893. Brauer. The study of the closely related Phyllopod Artemia was undertaken by the same author with the object of ascertaining whether reduction took place in parthenogenetically developing eggs.

The number of chromosomes in the germinal vesicle is eighty-four, and their structure is quadripartite, *i. e.*, each consists of four spheres. In the first maturation division two of these spheres are separated from the others. After this has taken place the maturation may proceed in two different ways. The second polar body may be formed and the elements of the dyad separated, or there may be an abortive attempt to form the second polar body, the chromatin, however, remaining undivided and the elements of the dyad not separated.

Cleavage and further development of the egg may take place in both of the above cases. In the first case it is necessary for this that the second polar body be drawn back into the egg, where it acts as would a male pronucleus. In the second case the nucleus left within the egg after the formation of the first polar body, becomes the cleavage nucleus. In the first case the somatic number of chromosomes is 168, in the second case 84.

It thus appears that the tetrads of the germinal vesicle are bivalent chromosomes and that the actual reduction may or may not take place.

1893. Moore published the results of his studies on the reproductive elements in Apus and Branchipus. With regard to Branchipus, the chief stress of the paper is laid upon the relation between karyokinesis and protoplasmic structure, the author believing "that the divisional phenomena of these cells are intimately related to a protoplasmic structure, which might be fitly described as 'Schaumplasma,' and one of the initial physical impulses toward metamorphosis is a fusion of some of the intra-nuclear globules; and a considerable portion of the complicated karyokinetic figures, with their centrosomes, pseudosomes and dictyosomes, appear to be the logical as well as the actual consequence of the continuance of this process."

The question of reduction is not entered upon in much detail. From the nucleus of the resting spermatocyte, however, are shown to arise ten chromosomes of dumbbell-shape. These become

arranged in the equatorial plate with the transverse constriction in the plane of the equator. This division consequently is apparently reducing. No longitudinal split is shown and the second spermatocytic division is very inadequately worked out.

b. Commentary.

Although agreeing with many points in the description of Gilson concerning the metamorphosis of the spermatids of Oniscus, my observations do not entirely coincide with his account of the earlier stages. The statement defining the most favorable season for obtaining preparations of the first stage does not hold true for the locality of Philadelphia, for I have sectioned material collected during every month of the year, except December and January, and have not found one month to be preferred over another with regard to the abundance of any particular stage.

I feel sure that the function of replacing the evacuated elements which he ascribes to the spermatogonia is the true one, but that their multiplication takes place by direct division I am unable to believe. On the one hand the weight of the evidence of modern research is against the occurrence normally of amitotic division in the germ cells. Moreover the work of vom Rath on Astacus creates a strong probability that the phenomena are similar in Oniscus. I have never seen amitotic division in the germ cells of Oniscus, and believe that the error arose from a failure to distinguish between the germ cells and the follicle cells. I cannot help a feeling of surprise that mitosis should have been so infrequently seen both by M. Gilson and his colleague, M. Carnoy. It is true that the mitoses of the spermatogonia are scattered, and occasionally no spindles at all will be met with in a follicle, but by cutting a sufficient number of sections cell division will be abundantly seen.

With regard to the question of reduction in the Crustacea, my results, much to my own surprise, do not coincide with those obtained by Rückert and vom Rath in the Copepods. The case in Cyclops is so clear that it seems to admit of no doubt, and its very clearness makes it probable that the divisions take place in a similar manner in a form so closely allied as Canthocamptus. The figures given by Häcker of this object do not, however, conclusively prove this to be the case, since the tetrads are cubical in shape, the length no greater

than the width. Indeed Häcker himself says of this object that it is not adapted to the solution of the problem of reduction. The like may be said of Artemia.

With Brauer's results on Branchipus, those obtained with Oniscus likewise do not agree. The double longitudinal split claimed by Brauer for the chromosomes of Branchipus is not shown in the figures with the clearness that might be desired. An oblique view of an elongated chromosome in Fig. 8 shows it to be split longitudinally, not twice but only once. In the absence of direct evidence to the contrary, the Figs. 8 and 9 might be explained equally well on the assumption that the twelve tetrads represent two univalent chromosomes longitudinally split and joined end to end.

In Oniscus, inasmuch as the first division separates two originally distinct chromosomes and the second presumably divides the chromatin longitudinally, the manner of reduction resembles that of Insecta as described by Henking (1890–'92), Paulmier (1899) and Montgomery (1898, '99).

If my interpretation of the method of reduction in Oniscus be correct, and that of Rückert concerning reduction in Cyclops be equally so, it becomes clear that the cell generation in which the true reduction takes place need not be the same for all members of a given class of animals. The order in which the reduction and equation divisions take place is, therefore, relatively unimportant; the significant thing, so far as our knowledge at the present day goes, appears to be that in the Arthropods both divisions should take place. Further research alone can show whether the apparent cases of transverse division in the first spermatocytes of Astacus, Crangon and Branchipus are really such. To the future must also be left the question as to which method of reduction, the Copepod or the Isopod type, is the rule among Crustacea.

M. Louise Nichols.

January 10, 1901.

Explanation of the Plates.

All of the figures, with the exception of 1, 2 and 68, are camera drawings made at the level of the microscope stage, and all except 3, 4*a*, 5, 6, 84–86, were drawn with a Zeiss homogeneous immersion objective $\frac{1}{12}$, ocular No. 6, tube length 100 mm. In those marked * the chromosomes are not all shown.

Plate XI

1. Free-hand drawing to illustrate the male reproductive organs of one side. *a*, *b*, *c*, lobes of the testis; *v*, vas deferens; *p*, penis; *x*, suspending tissue.
2. Lobes of the testis in longitudinal section (semi-diagrammatic). *a*, *b*, *c*, as before; *spg.*, spermatogonia; *f. c.*, follicle cell.

Plate XII

3. Longitudinal section of the vas deferens (Zeiss ocular 4, obj. AA). *b*, *c*, lobes of the testis.

4*a*. Small portion of the wall of the anterior region of the vas (oc. 6 obj. D).

4*b*. Secretory cell from the anterior region of the vas.

Plate XIII

5. Small portion of a testis lobe in longitudinal section. *f. c.*, follicle cells; *m. l.*, muscular layer (oc. 6, obj. D).
6. Cells from the suspending tissue (*cf.* Fig. 1, x) (oc. 6, obj. D).
7. (*a*) Spermatogonium in an early spireme stage; *ncl*, nucleolus. Centrosomes beginning to divide. (*b*) Resting spermatogonium with large masses of chromatin, probably beginning to degenerate.

*8, *9, 10. Later spireme stages.

11. (*a* and *c*) Resting spermatogonia. (*b*) Spireme beginning to segment.
12. Equatorial plate in side view.
13. (*a*) Equatorial plate in pole view. (**b*) Spermatocytic prophase. *m. l.*, muscle layer.
14. Equatorial plate in side view, showing the longitudinal split in the chromosomes.

*15. Metaphase.

16. Approximate pole view of a stage similar to 15.
17. Anaphase.
18. Late anaphase. Mid-body.

*19, *20. Reconstruction of the daughter nuclei. In 20 the mid body has migrated to the periphery.

*21. (*a* and *b*) Reconstruction of the daughter nuclei. (*c*) Degenerating spermatogonium.

Plate XIV

22, 23–28. Synapsis. 26. 1–16, chromosomes.
27. Different forms of the chromosomes in the synapsis.
29. Formation of the nuclear membrane.
30. Resting spermatocyte. *spg.*, spermatogonium.
31. Irregular arrangement of the nuclear network, occasionally seen just before the formation of the nuclear membrane.

PLATE XV

32, *33–47. Prophases of the first spermatocyte. 44. 1–16, chromosomes.
46 and 47. Strongly decolorized sections showing the longitudinal split of the chromosomes. Divergence of the centrosomes.
48. (*a*) Side view of the equatorial plate of the first spermatocyte. (*b*) Anaphase of the first spermatocyte.
49–53. Equatorial plate of the first spermatocyte in side view.
54. Chromosomes of the first spermatocyte, showing the longitudinal split.
55, 56. Pole views of the equatorial plate of the first spermatocyte.
57. Slightly oblique view of the same.

PLATE XVI

58. Anaphase (side view).
59. Anaphase (pole view).
60. Anaphase (tangential sections).
61. Telophases.
62. Side view of the equatorial plate of the second spermatocyte.
63. The same more strongly decolorized.
64. Pole view of the same.
65. Metaphase.
66, 67. Telophases.
68. (*a* and *b*) Mode of formation of the two main types of chromosome in the first spermatocyte. (*c*) Intermediate form.
69, 70. Reconstruction of the nucleus of the spermatid.
70. Disappearance of the cell boundaries. *x*, remains of degenerated cells.
71. Variation in the appearance of the spermatid nucleus.
72. Commencing elongation of the spermatid nucleus.
73. Group of spermatids from near the margin of the testis lobe. Appearance of cytoplasmic striations (hæmatoxylin and Bordeaux red).

PLATE XVII

74–79. Further development of the spermatids.
74. Stage succeeding 72. (*a*) Longitudinal; (*b*) transverse section (iron hæmatoxylin).
75. Longitudinal section (iron hæmatoxylin).
76. Longitudinal section (Biondi-Ehrlich). The middle figure alone is complete anteriorly.
77. (*a*) Longitudinal; (*b*) oblique section (iron hæmatoxylin).
78, 79. Nearly mature sperm colonies in incomplete longitudinal section (hæmatoxylin and Bordeaux red).
80. Cross sections at different levels of nearly mature sperm colonies. (*a*) anterior to the nuclear region; (*b*, *c* and *d*) nuclear region; (*e*), posterior to nuclear region (saffranin and malachite green, black = red, gray = green).

PLATE XVIII

81. Cross sections of colonies of about the same stage (iron hæmatoxylin).
82. Cross sections of colonies at a later stage (iron hæmatoxylin).
83. Group of spermatids with convoluted nuclei. Cytoplasm of the individual cell still evident (iron hæmatoxylin).
84. Mature sperm colony (Delafield's hæmatoxylin) (oc. 6, obj. D).
85. The same (hæmatoxylin and Bordeaux red).
86. The same (oc. 4, obj. AA).

LITERATURE LIST.

NOTE.—Those papers marked * I have not had an opportunity of examining.

1895. Auerbach, L. Spermatologische Mittheilungen: 72 Jahr. Schles. Gesell. f. Vaterl. Cult.-Zool. bot. Sect., pp. 30–34.

1895. Ballowitz, E. Die Doppelspermatozoa der Dytisciden: Z. w. Z., XLV.

1894. Ballowitz, K. Zur Kenntniss der Samenkörper der Arthropoden: Int. Monat. Anat. u. Phys., 11.

1892. Brauer, A. Das Ei von Branchipus Grübii von der Bildung bis zur Ablage: Abh. preuss. Akad. Wiss.

1893. Brauer, A. Zur Kenntniss der Reifung des parthenogenetisch sich entwickelnden Eies von Artemia salina. A. m. A., XLIII.

1885. Budde-Lund, G. Crustacea Isopoda Terrestria.

1885. Carnoy, J. B. La cytodiérèse chez les Arthropodes: La Cellule, Vol. I.

*1883. Friedrich, H. Geschlechtsverhältnisse der Onisciden: Zeit. Naturwiss.

1882. Gerstaecker, A. Bronn's Klassen u. Ord. des Thierreichs Fünfter Band, II Abth.

1884–'86. Gilson, G. Spermatogénèse chez les Arthropodes: La Cellule, Vols. I and II.

1878. Grobben, C. Beiträge zur Kenntniss der männlichen Geschlechtsorgane der Dekapoden: Arb. Zool. Inst. Wien.

1890. Häcker, V. Ueber die Reifungsvorgänge bei Cyclops: Z. A., pp. 551–558.

1892. Häcker, V. Die Eibildung bei Cyclops und Canthocamptus: Zool. Jahr., V.

1895. Häcker, V. Die Vorstadien der Eireifung: A. m. A., XLV.

1890–'92. Henking, H. Untersuchung über die ersten Entwicklungsvorgänge in den Eiern der Insekten: Z. w. Z., XLIX, LI, LIV.

1883. Herrmann, G. Sur la Spermatogénèse des Crustacés podopthalmes. C. R. de l'Akad. des Sciences, 97, p. 958.

1883. Herrmann, G. Sur la Spermatogénèse des Crustacés édriopthalmes. C. R. de l'Akad. des Sciences, 97, p. 1009.

1898. Hoffmann, R. W. Ueber Zellplatten und Zellplattenrudimente: Z. w. Z., LXIII.

1892. Ishikawa, M. Studies on the Reproductive Elements: Jour. Coll. Sci. Imp. Univ. Japan, Vol. V.

*1895. Mari, M. Sopra la regenerazione delle spermatogonio nei crostacei Decapodi: Bull. Soc. Ent. Ital. Anno 26, pp. 396–407.

*1895. Mari, M. Canateri delle cellula seminali nel Granchio di Fiume. Contribuzione alla Istologia del vesticolo dei Decapodi brachiuri. Ibid. Anno 27, pp. 3–10.

1898. Meves, F. Zelltheilung: Merkel u. Bonnet Erg., Anat. Hefte.

1898. Montgomery, T. H., Jr. The Spermatogenesis of Pentatoma, etc. Zool. Jahrb., XII.

1899. Montgomery, T. H., Jr. Chromatin Reduction in the Hemiptera, a Correction. Zool. Anz., Bd. XXII, No. 580.

1893. Moore, J. E. S. Some Points in the Origin of the Reproductive Elements in Apus and Branchipus. Q. J. Mic. Sc., XXXV.

1889. Müller, G. W. Die Spermatogenese der Ostracoden: Zool Jahrb. 3. Anat. Hefte., p. 677.

1884. Nussbaum, M. Ueber Veränderungen der Geschlechtsproducte bis zur Eifurchung: A. m. A., Bd. 23. (Astacus.)

*1890. Nussbaum, M. Studien an californischen Cirrhipedien Bonn.

1899. Paulmier, F. C. The Spermatogenesis of Anasa tristis: Am. J. M., XV. Suppl.

1891. vom Rath, O. Ueber Bedeutung der amitotische Kerntheilung von Hoden: Z. A., 14, pp. 342–363. (Astacus.)

1895. vom Rath, O. Neue Beiträge zur Frage der Chromatinreduktion in den Samen-und Eireife: A. m. A., 46, p. 168.

1900. Richardson, Harriet. Synopsis of North American Invertebrates: Amer. Nat., April, p. 303.

1894. Rückert, J. Die chromatinreduktion bei der Reifung der sexualzellen: Merkel u. Bonnet Ergeb. d. Anat. u. Entwick., Anat. Hefte. 2re Abt. III, pp. 544–548.

1894. Rückert, J. Zur Eireifung bei Copepoden: Merkel u. Bonnet, Anat. Hefte, 1 Abt., XII Heft.

1885. Sabatier, A. Sur la spermatogénèse des Crustacés Décapodes. C. R., 100, p. 391.

*1892. Sabatier, A. De la spermatogénèse chez les Crustacés Décapodes. Nac. Inst. d. Zool. d. Montpellier, S. 2, Mem. 3.

1895. Steuer, A. Zoologische Ergebnisse VI. Sapphirinen des Mittelmeeres und der Adria, etc.: Denkschr. Akad. Wien, Math. Nat. C. 62.

1886. Stuhlmann, F. Beiträge zur Anatomie der inneren männlichen Geschlechtsorgane und zur Spermatogenese der Cypriden. Z. w. Z., 44, p. 536.

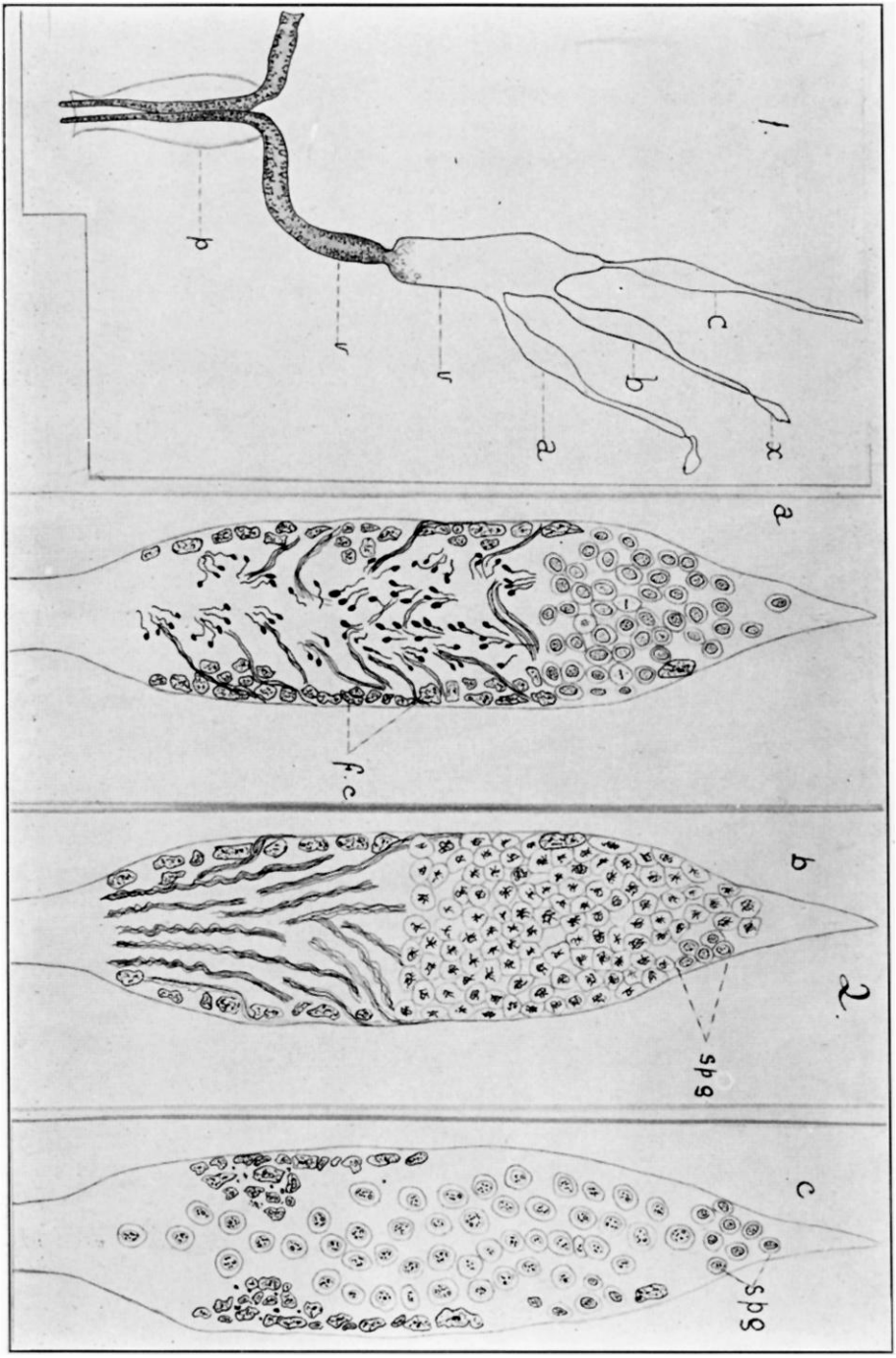
1886. Wielowieyski, H. de. Spermatogénèse des Arthropodes: Archiv. Slavs de Biologie, T. II, p. 33.

1895. Wilcox, E. V. Spermatogenesis of Caloptenus and Cicada. Bull. Mus. Comp. Zool. Harvard College, Vol. XXVII, No. 1.

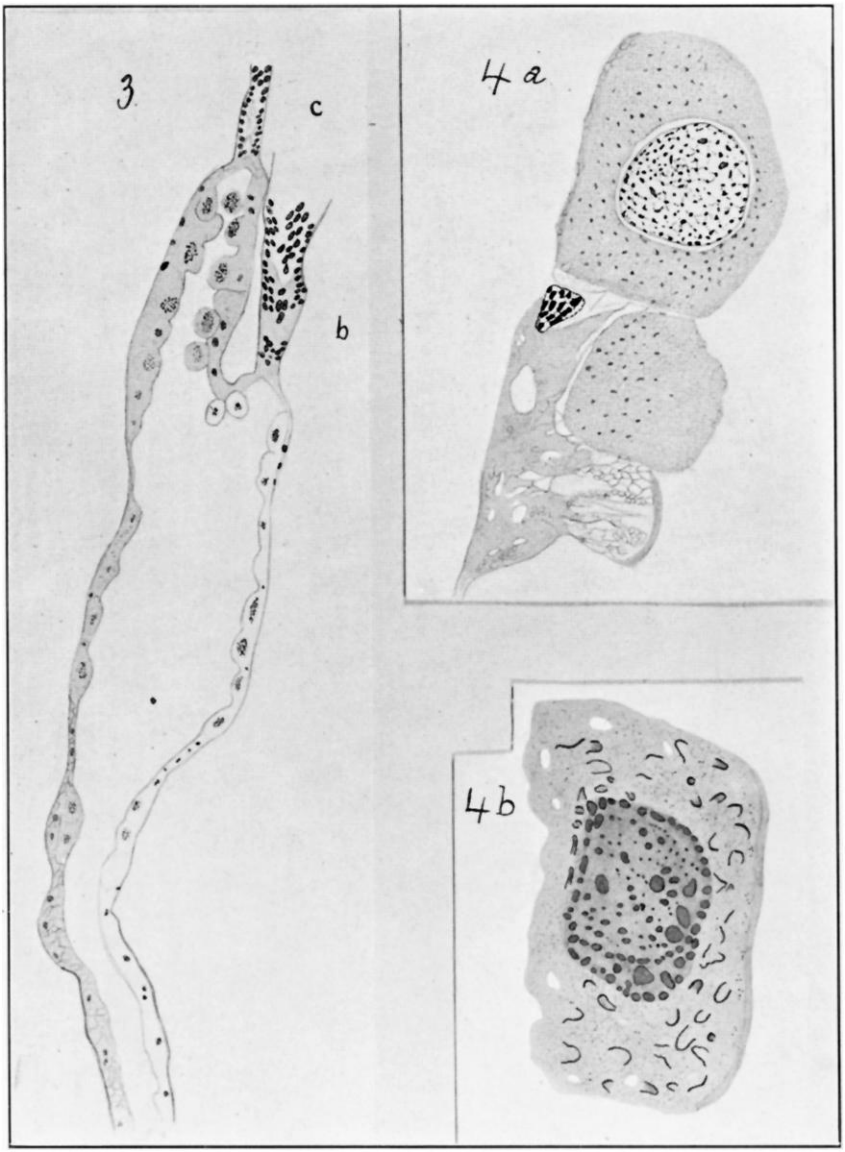
1900. von Winiwarter, H. Le Corpuscle Intermediaire et le nombre des Chromosomes du Lapin: Arch. de Biol., T. 16.

1898. Woltereck, R. Zur Bildung und Entwicklung des Ostrakoden-Eies: Z. w. Z., 64, p. 596.

1885. Zacharias, O. Ueber die amäboiden Bewegungen der Spermatozoen von Polyphemus pediculus: Z. w. Z., XLI.



NICHOLS—SPERMATOGENESIS OF ONISCUS ASELLUS LINN.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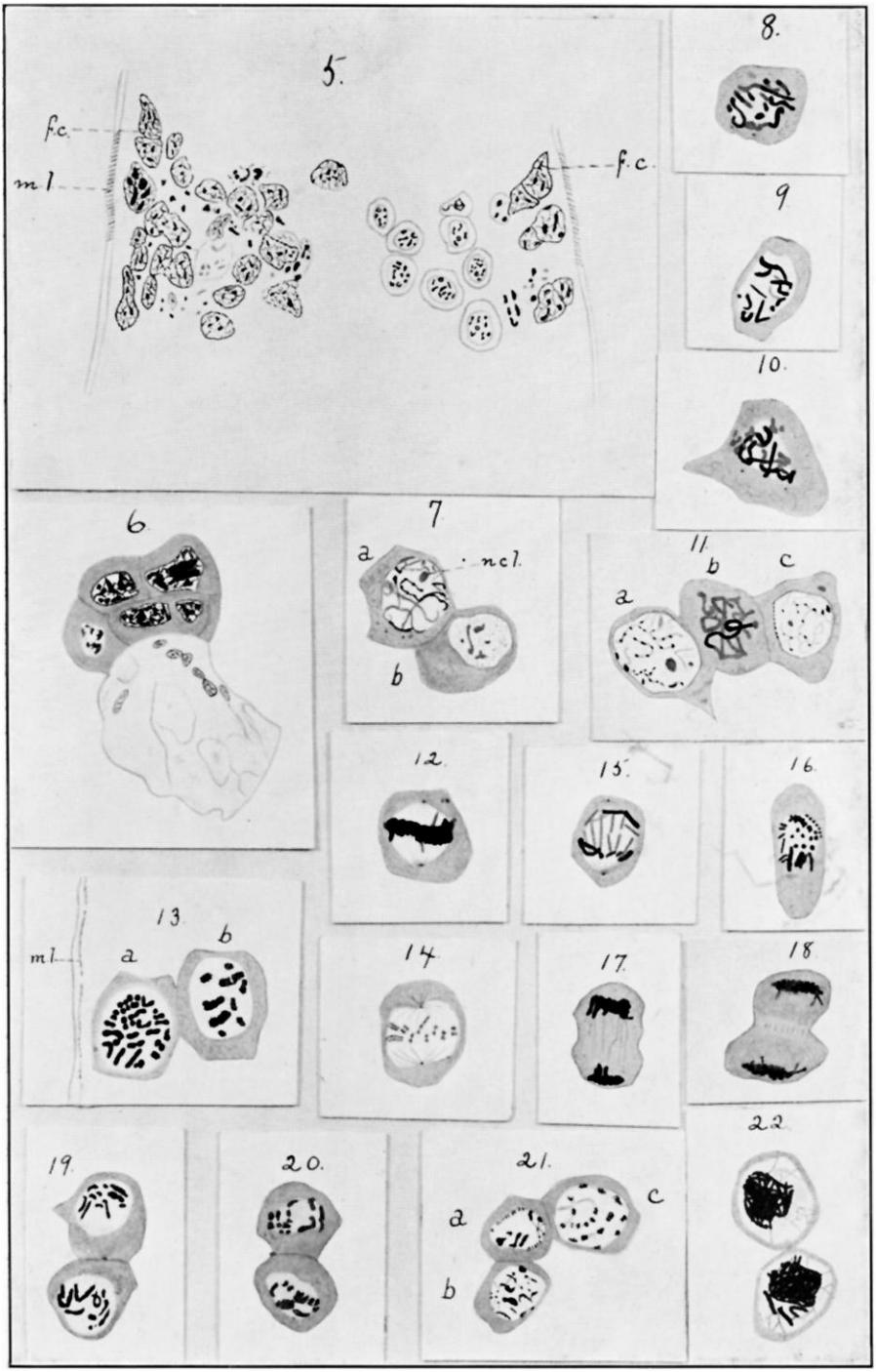
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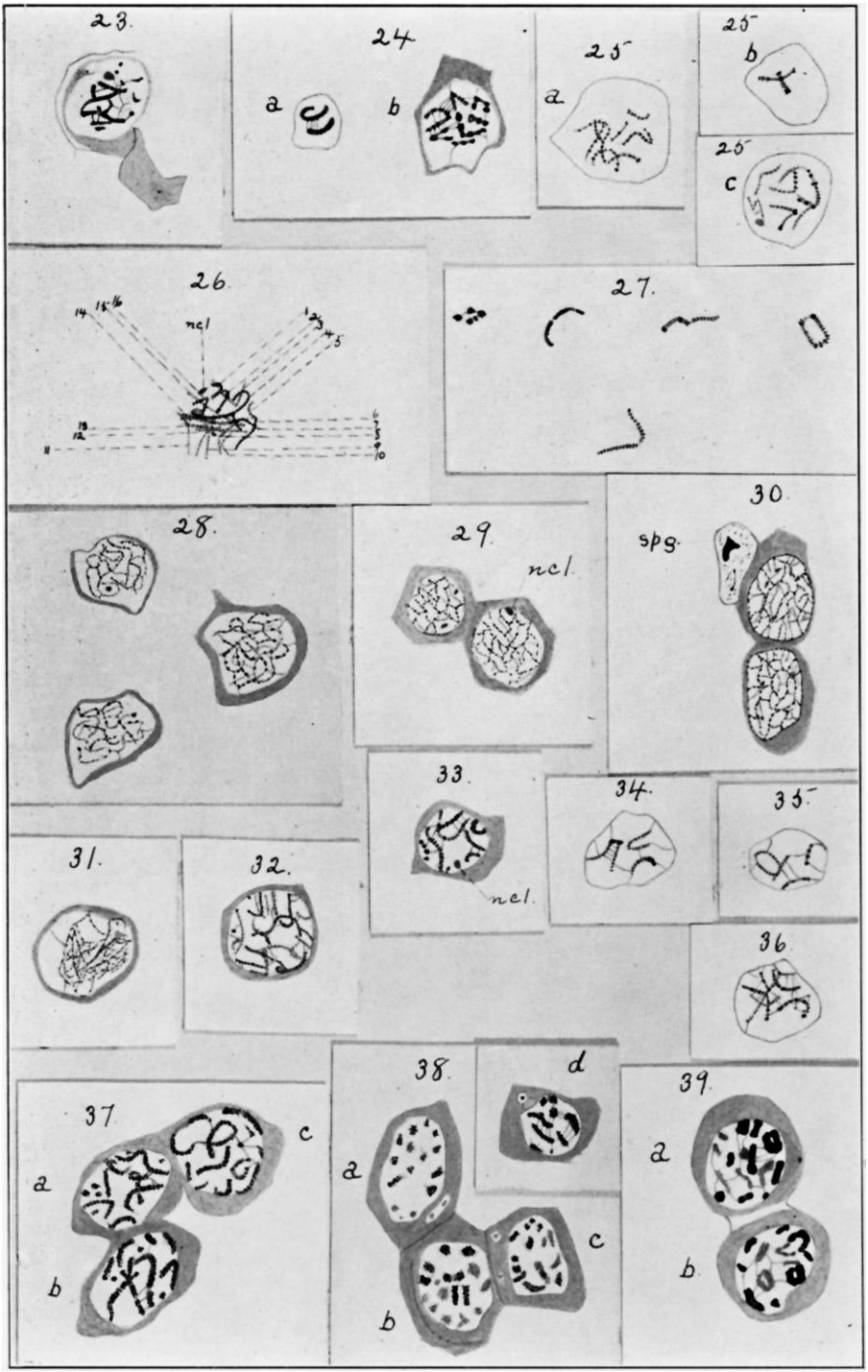
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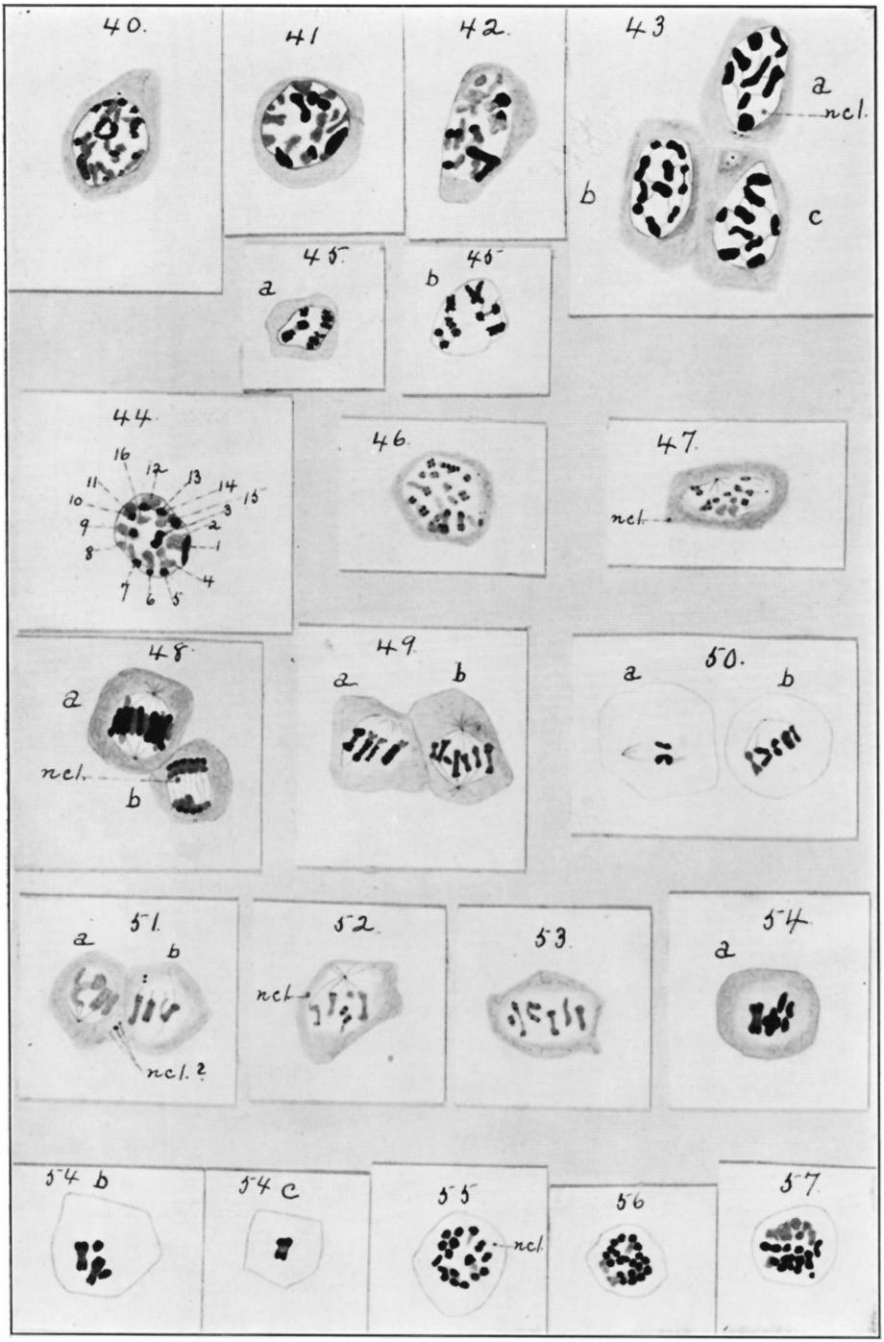
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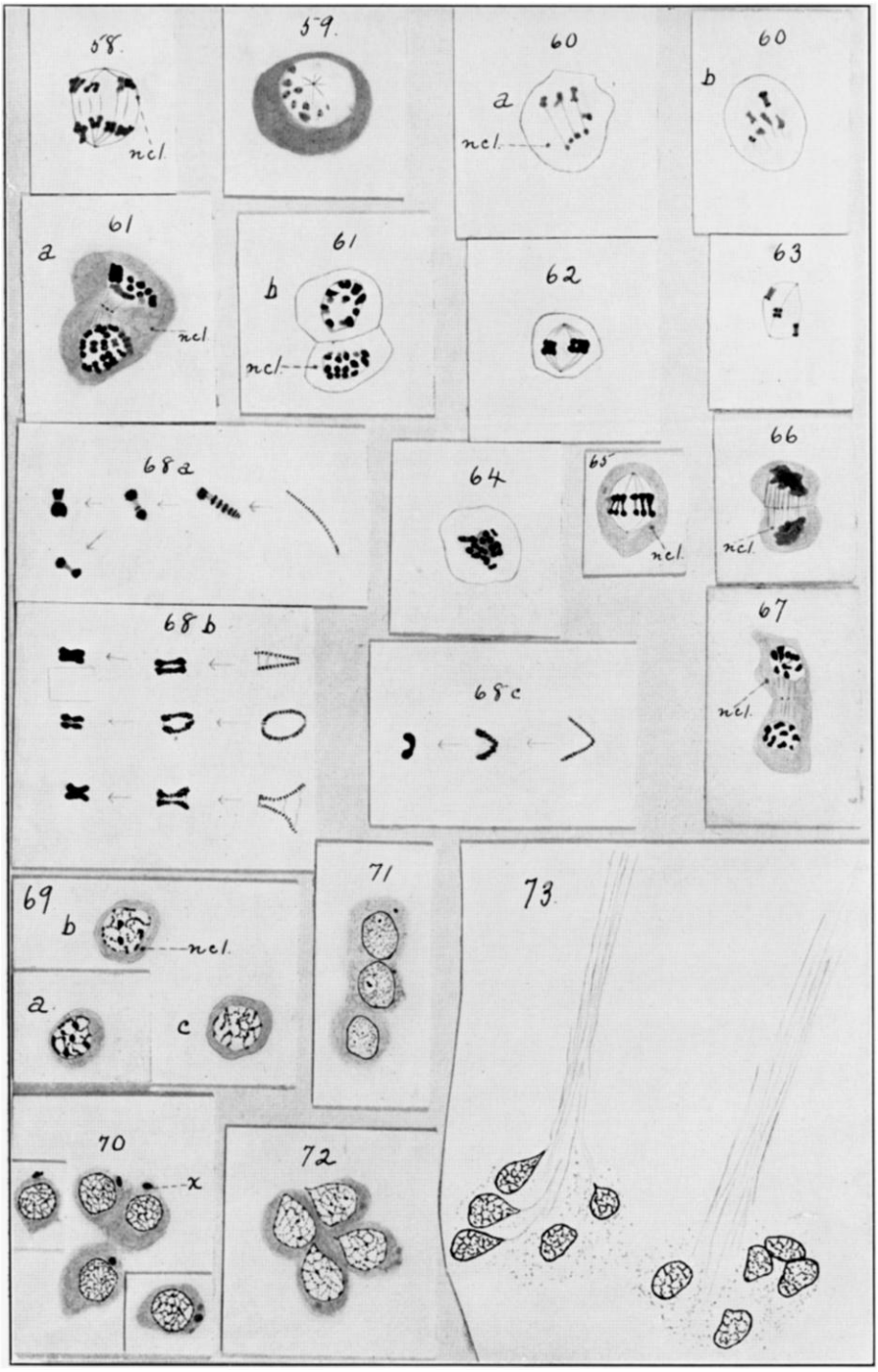

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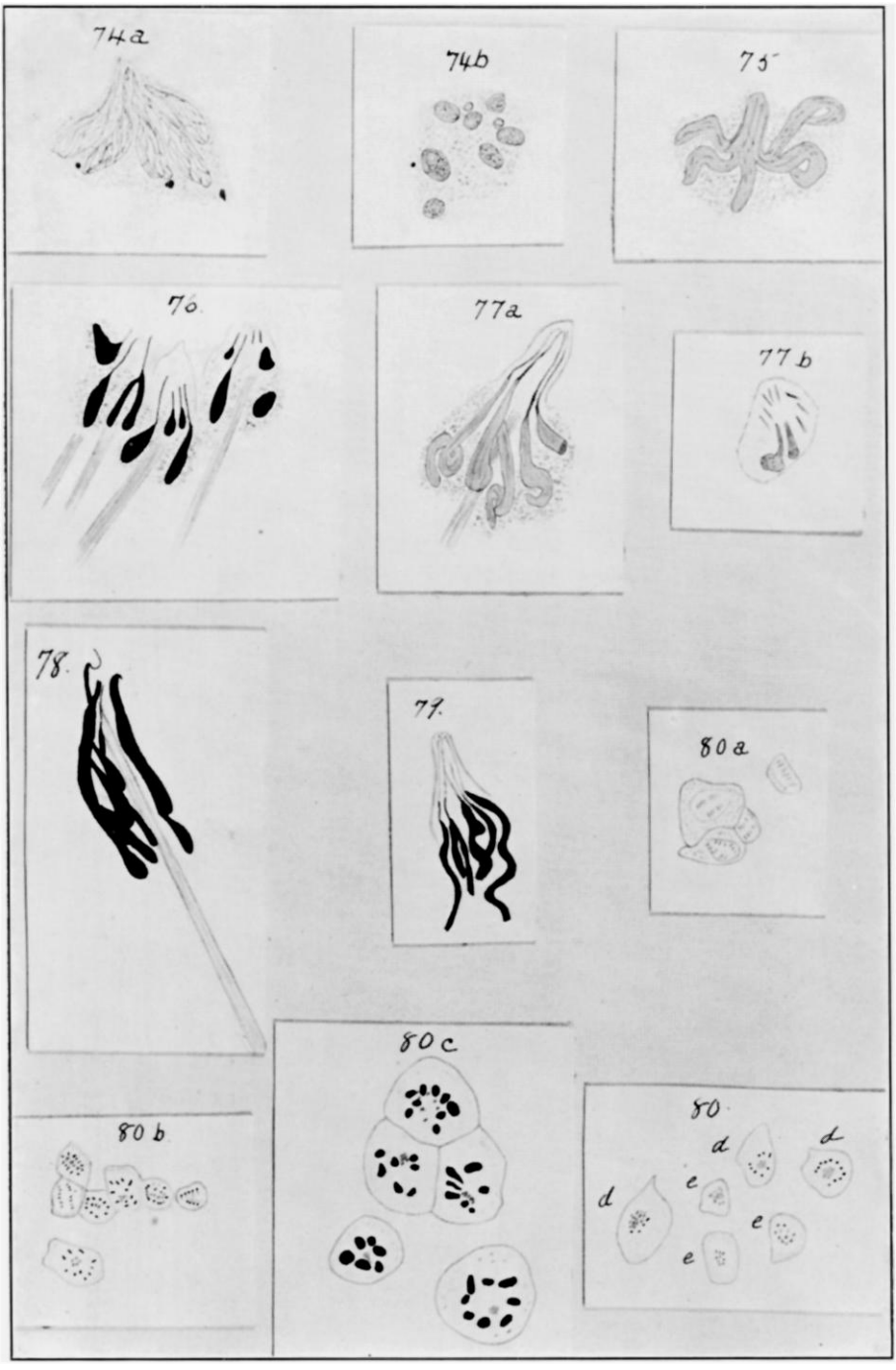

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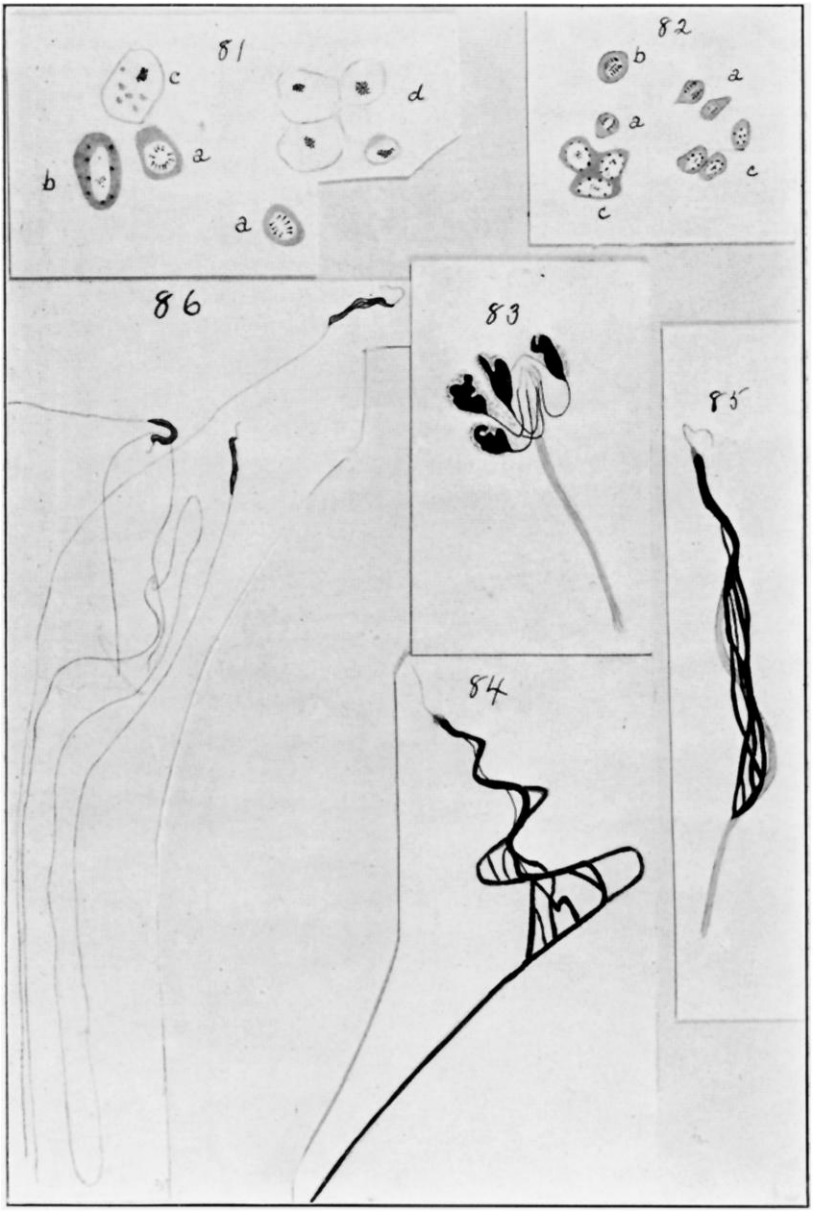

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